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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

A process for the preparation of a Medicinal Agent for the Treatment of Hypercholesterolemia from the Oil of Marine Animals

We, TAKEDA CHEMICAL INDUSTRIES LTD. of 27, Doshomachi 2 - Chome, Higashiku, Osaka, Japan, and RIKEN VITAMIN OIL CO. LTD., of 2 Shiba-Minami-Sakumacho-1 Minatoku, Tokyo, Japan, both joint stock companies organised under the laws of Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a process for the preparation of a medicinal agent for the treatment of hypercholesterolemia from the oil of marine animals.

Arteriosclerosis is of a wide variety according to the differences in the situation of development of the disease and the condition of the sclerosis. Atherosclerosis is mainly characterised by the thickening of the intima of aorta of coronary arteries and of cerebral arteries accompanied by the deposit of lipids thereonto. Recently, atherosclerosis became one of the worst diseases for modern times.

Etiological progressive factors of atherosclerosis are many and varied, so that the prevention and therapy of the disease are very complicated matters. It has been widely recognised that an abnormal metabolism of lipids plays an important role in the development and progression of this arteriosclerosis and that an abnormal metabolism of cholesterol in particular is a main cause of the disease. In these circumstances, there have been used various medicinal preparations which aim to improve the abnormal metabolism of lipids and particularly reduce the content of cholesterol in blood. Among these preparations, the unsaturated fatty acids have been hitherto considered a most suitable and natural medicine, because they are natural products and free from any considerable side-effect.

Since the 1920's, some of the unsaturated fatty acids containing more than one double-bond in their molecule have been regarded as the necessary fatty acids. Recently, it has, however, been thought that they reduce the content of cholesterol in serum, but their action cannot be yet explained completely. In the experiments using animals, it has been observed that the unsaturated fatty acids behave in a different way with a different kind of animal. However, the previous researches have proved that the unsaturated fatty acids are also capable of reducing the hypercholesterolemia in human beings. The mechanism for this may be explained as follows:

a) The unsaturated fatty acids will enhance the metabolism of cholesterol (however, some reports have stated that the synthetic unsaturated fatty acids will hinder the metabolism of cholesterol) and particularly promote the catabolism of cholesterol, namely its conversion to bile acids in liver. The bile acids are subsequently excreted from the human body, so that the cholesterol is discharged out in this way.

b) Two-thirds of the content of cholesterol in serum are present in a form of its ester with the fatty acids. As compared to an ester of cholesterol with a saturated fatty acid, an ester of cholesterol with an unsaturated fatty acid is much more easily produced and shows a lower melting point as well as a higher solubility in the serum, so that it is much more difficult to deposit onto the cells of the blood vessel walls. Moreover, its conversion to bile acids is much easier.

c) The unsaturated fatty acids participate in the synthesis of phospholipids which in turn, play an important role in the combustion of fat as well as in the colloidization and stabilisation of cholesterol in the serum.

Vegetable oils mainly comprising linoleic acid, one of the diethenoic acids, are widely used at present, and it has recently been

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found that the much more highly unsaturated fatty acids than linoleic acid are also effective.

It is generally said that the activity of polyethenoic acids as essential fatty acids is increased in the sequence of linoleic acid (diethenoic acid), arachididonic acid (tetraethenoic acid) and docosahexaenoic acid (hexaethenoic acid) with increased degree of unsaturation, and that the ratio of activity of the aforesaid three acid compounds is 1:3.5:5. Recent research of human beings has proved that substantially the similar relationship of the activities of the above-mentioned acid compounds also exists with respect to their actions of reducing the content of cholesterol in blood.

Although linolenic acid (tri-ethanoic acid) and clupanodonic acid (penta-ethenoic acid) show a markedly decreased biological activity due to the difference in the positions of their double-bonds which are a source of the activity the activities of them can be enhanced by combination with linoleic acid, arachidonic acid or docosahexaenoic acid. Further, it has been said that the ratio of the activity of a mixture of linoleic acid and arachidonic acid to the activity of linoleic acid alone is 6.2:1. Accordingly, it may be observed that there has occurred synergistic effect in the activity of the unsaturated fatty acids owing to a combined use of them.

Thus, it is reasonable to use the polyethenoic acids as the essential fatty acids or as the medicinal agent to reduce the content of cholesterol in the serum. In this case, however, a precaution should be paid to the presence of peroxides in the unsaturated fatty acid preparations. That is, if the unsaturated fatty acids containing large amounts of the peroxides are administered, it is often observed that the content of cholesterol in the serum is increased and that the increase in the body weight is suppressed. On the other hand, the peroxides themselves are not only toxic but also would degrade the valuable substances such as vitamins which are present in foodstuff. These peroxides have been derived from the very unstable double-bonds of the unsaturated fatty acids which serve also as the source of the biological activities of the aforesaid acids. These peroxides would also involve autoxidation, isomerization, polymerization and/or formation of ring compounds. Accordingly, fish oil, which is one of the naturally occurring sources for the highly unsaturated fatty acids, has never been used without further processing such as hydrogenation. A further reason for the processing of the fish oil is the undesirable odour of the fish. The peroxides exhibit a marked action of denaturation of protein and an action of inhibition of the enzyme activity, and they would destroy rats.

An object of the present invention is to produce a cheap medicinal agent for the treatment of hypercholesterolemia, by electrolytically treating the oil of marine animals containing large amounts of the highly unsaturated fatty acids, but a small amount of cholesterol, so as to reduce the secondarily formed peroxides as well as other harmful substances from the oil without changing the essential nature of the oil itself.

According to the present invention, there is provided a process for the preparation of a medicinal agent for the treatment of hypercholesterolemia from the oil of marine animals, characterised in that the oil of marine animals is supplemented with an antioxidant and then purified by electrolytically reducing it in the presence of a neutral or alkaline solution of an electrolyte and that the purified and stabilized oil is then used as the base material to prepare the medicinal agent in a conventional manner.

The electrolytic reduction of the marine animal oil is carried out in the presence of the above-mentioned electrolyte solution as well as in the presence of a powder of a metal selected from aluminium, magnesium or zinc. In this way, the purification of the marine animal oil can be performed more effectively than in the absence of the metal powder.

As the oil of marine animals which may be used in the invention, there may be mentioned sardine oil, herring oil, bonito oil, whale oil, mackerel oil, saury pike oil and oil of the internal organs of cuttlefish as well as residual oils which are obtained in the production of vitamin oils, such as liver oils of pollack, shark, cod, tunny and whale.

The solutions of the electrolyte which may be used in the invention are as follows:

When an alkaline electrolyte solution is required, there is employed an aqueous solution of an inorganic base such as an alkali metal hydroxide or an alkali metal carbonate or of a salt of an organic acid such as, for example, sodium acetate. When a neutral solution is required, an aqueous solution of, for example, sodium chloride or sodium sulfate is used so as to obtain a good electric conductivity.

Furthermore, the antioxidants which may be used in this invention include N.D.G.A. (nordihydroguaiaretic acid), propyl gallate, B.H.A. (butylated hydroxyanisole), B.H.T. (butylated hydroxytoluene), and α-tocopherol.

On carrying out the electrolytic reduction of the oil according to the present invention, it is preferable to employ an electrolytic cell in which a diaphragm is provided between the anode chamber and the cathode chamber which is to be charged with the oil of marine animals previously containing the antioxidant. It is preferable that the cathode be constituted from a metal selected from Zn, Pb, Sn, or Cd, and that the reduction be performed while agitating the catholyte in such a way that a

suspension is formed.

In accordance with the present invention, the peroxides and other harmful substances which have been secondarily formed in the oil of marine animals, for example, the fish oil or liver oil containing large amounts of the highly unsaturated fatty acids, can be substantially removed from the oil through the action of the nascent hydrogen which is produced in the electrolytic reduction, and decoloration and partial deodorization of the oil can also be achieved, so that there is obtained a purified oil which is light-colored and substantially free from the fish odour. In this case, of course the highly unsaturated fatty acids, which constitute the essential material of the oil have not been subjected to any degradation. The purified oil is then used as the base material to prepare the medicine for the treatment of hypercholesterolemia in a conventional manner. The medicine may be in any form of capsule, oily preparation, emulsion or gelatin-coated granule. Highly unsaturated fatty acids should however be shut off from air in order to prevent their oxidation.

For instance, the purified oil may be conveniently mixed with vitamin B_6 palmitate, inositol, α-tocopherol and an emulsifier and the mixture may be then emulsified by means of a homogenizer to give an emulsion. The purified oil may also conveniently be mixed with vitamin B_6 palmitate and an emulsifier to give a paste which may be then placed in capsules.

Moreover, the purified oil may be conveniently shaped into the gelatin-coated granules by dispersing said purified oil and vitamin B_6 palmitate etc. in a coating solution such as gelatin solution with the aid of an emulsifier, injecting the resulting dispersion into a mass of a water-repellent substance such as starch esters in a rotary drum fitted with stirring blades, drying the mixture and sieving the resulting powder, or alternatively suspending said dispersion in a mineral or vegetable oil with strong agitation, cooling the resulting suspension to coagulate it, filtering the coagulated particles, washing with a solvent and drying them.

Since the medicinal agent obtained by the process of the invention is prepared by using as the base material the purified oil which has been made to contain a substantially reduced amount of the peroxides and other harmful substances, the medicinal agent obtained according to the invention is superior to such an agent which has been prepared from the non-purified oil.

Two experiments were carried out by using the purified oil which was prepared by purifying the residual oil obtained in the molecular distillation of cuttlefish oil and liver oil of pollack, as well as the non-purified raw oils of the cuttlefish and pollack. The test animal was rats (Sprague-Dawley strain, male, four-weeks-old). One test consisted of five rats. Test procedure was as follows: After the control animal had been fed only the undermentioned basic diet for two to six weeks the amount of increase in the body weight and the content of the cholesterol in serum of the control animal were determined and compared to those of the test group of rats which was fed with the test diet. The blood samples were taken by decapitation of the rats. The determination of the cholesterol content was carried out by the Abell method.

BASIC DIET:

This had the following composition:

Casein	15%	Cholesterol	1.0%
Hydrogenated oil	10%	Sodium chlolate	0.2%
Granulated sugar	67.1%	Choline chloride	0.12%
Salt mixture	4%	Vitamin mixture	0.5%
Pulverized filter paper	2%		

Test diet:

In the first experiment, the test diet used was the same as the above-mentioned basic diet except that the content of the hydrogenated oil was reduced to 5% and 5% of the test oil was instead added thereto. In the second experiment, the test diet used was the same as the aforesaid basic diet except that the content of the hydrogenated oil was reduced to 5%, 7.5%, 9.0% and 9.0% respectively and 5%, 2.5%. 1% and 1% of the test oil was instead added thereto, respectively, the total amount of the hydrogenated oil plus the test oil being 10% of the composition of the test diet in each case.

FIRST EXPERIMENT

Test Group No.	Test Oil	
1	Cuttlefish oil	5%
2	Purified cuttlefish oil	5%
3	Residual pollack oil	5%
4	Purified residual pollack oil	5%

SECOND EXPERIMENT

Test Group No.	Test Oil	
1[1]	Purified cuttlefish oil	5%
2[1]	"	2.5%
3[1]	"	1.0%
4[1]	Safflower oil	1.0%

Results of Experiments

When the rats were fed the above-mentioned basic diet which contained a low amount of protein, saturated fat, cholesterol and cholic acid, it was observed that the cholesterol content in serum increased apparently after about the second week and reached a value of about 5 to 6 times as much as the normal value in the fourth week. The activity of the unsaturated fatty acids for reducing the content of cholesterol could be observed after the fourth week, as is clear from the following two Tables, which show the results of the experiments.

FIRST EXPERIMENT

Test Group No.	Number of Rats	Amount of Increase in Body Weight (g.)	Content of Cholesterol in serum (mg %)
1	5	56	269
2	5	68	207
3	5	59	235
4	5	70	185
Control	5	78	443

SECOND EXPERIMENT

Test Group No.	Number of Rats	Amount of Increase in Body Weight (g.)	Content of Cholesterol in serum (mg %)
1[1]	5	62	149
2[1]	5	55	161
3[1]	5	65	163
4[1]	5	74	179
Control	5	58	351

As will be clear from the above-mentioned results, it is apparently noted that the purified oil has a higher activity than the untreated oil with respect to the reduction of the content of cholesterol in serum without suppressing the normal increase in the body weight, and also that the activity of the purified oil is not inferior to that of safflower oil.

In further experiment using untreated fish oil which has been stored for a long period of time, it was found that the amount of the diet taken in by rats was considerably decreased due to its unfavourable odor. In this respect, the invention shows an advantageous effect, too, namely there is not observed any decrease in the intake-amount of the diet which contains the purified oil according to the present invention.

A further advantage of the invention is that the content of the active ingredient in the purified oil is higher than in the vegetable oils such as safflower oil, maize oil, sunflower oil and cotton seed oil, which are conventionally used. Although these vegetable oils comprise linoleic acid as the main ingredient, they further contain considerable quantities of oleic acid and saturated fatty acids which have been said to antagonize the activity of the linoleic acid for reducing the content of cholesterol. On the other hand, a report has stated that 16 g. of ethyl linoleate are essential to a man in a day even in a pure state. If this report is taken into consideration, it follows that administration of much larger amount of the linoleate is necessary to be effective for the reduction of the cholesterol content. As can be seen in many reports, administration of 30 to 50 g. of the linoleate often causes gastroenteric intolerance, nausea and vomiting, and this inevitably leads to the undesirable effects which usually result from the intake of high calories. While, the preparation produced by the process of the invention even in a lower dosage may serve for the treatment of hypercholesterolemia.

The invention will now be illustrated with reference to the following Examples.

EXAMPLE 1

To 200 g. of the oil of the internal organs of cuttlefish of a peroxide value of 18 and an iodine value of 182 are added 0.05% of butylated hydroxyanisole and the mixture is charged together with 200 g. of a solution 5% of sodium hydroxide into the cathode chamber of an electrolytic cell fitted with the diaphragm and then electrolytically reduced in the state of a suspension under stirring by means of a direct current of 7 amp/dm^2 at a voltage of 5 V. After 2 hours, the oil is removed and washed three times with hot water at 80°C. The resulting purified oil shows an peroxide value of 4.0 and an iodine value of 180. Yield is 187 g. The purified oil obtained is used as the base material and mixed with proper proportions vitamin B_6 palmitate and glyceryl monostearate as the emulsifier to give a paste which is then processed to the form of a capsule.

EXAMPLE 2

To 200 g. of the residual oil of pollack liver oil of a peroxide value of 6.5 and an iodine value of 148 are added 0.05% of butylated hydroxytoluene and then treated in a similar way to Example 1, except that 5% by weight of zinc powder are further added to the electrolyte solution. The resulting purified oil has a peroxide value of 3.2 and an iodine value of 149. Yield is 185 g. This purified oil is used as the base material and mixed with proper proportions of lecithin, inositol, vitamin B_6 palmitate, and perfume, to give an oily preparation.

EXAMPLE 3

To 200 g. of the residual oil of shark liver oil of a peroxide value of 5.8 and an iodine value of 148 are added 0.03% of nordihydro-

guaiaretic acid and 0.02% of butylated hydroxytoluene and then the mixture is electrolytically reduced in a similar way to Example 1 using 200 g. of an aqueous solution of 7% of sodium chloride as the electrolyte. The purified oil obtained shows a peroxide value of 2.8 and an iodine value of 147. Yield is 180 g. The purified oil is used as the base material and mixed with proper proportions of vitamin B_6, water and emulsifiers such as glyceryl monostearate and sucrose monostearate, and the mixture is then emulsified in a homogenizer to give an emulsion.

EXAMPLE 4

To 200 g. of mackerel oil of a peroxide value of 6.9 and an iodine value of 165 are added 0.02% of butylated hydroxytoluene and 0.03% of butylated hydroxyanisole, and then the mixture is treated in a similar way to Example 1 using 10 g. of zinc powder together with 200 g. of an aqueous solution of 7% of sodium hydroxide as the electrolyte.

The purified oil obtained shows a peroxide value of 3.4 and an iodine value of 166. Yield is 188 g. This purified oil is used as the base material and mixed with proper proportions of inositol, vitamin B_6 palmitate and an emulsifier glyceryl monostearate to give a paste which is then processed to the form of a capsule.

EXAMPLE 5

To 200 g. of the residual oil of pollack liver oil of a peroxide value of 6.5 and an iodine value of 148 are added 0.05% of butylated hydroxytoluene and then the mixture is treated in a similar way to Example 1 except that 5% by weight of zinc powder are further added to the electrolyte solution.

The purified oil obtained shows a peroxide value of 3.2 and an iodine value of 149. Yield is 185 g. This purified oil is used as the base material and mixed with proper proportions of vitamin B_6 palmitate and an emulsifier such as glyceryl mono-oleate. The resulting mixture is then admixed with an aqueous solution of 50% of gelatin-sugar mixture under stirring. The dispersion obtained is then suspended in a soy bean oil in the form of fine particles under strong agitation, and the temperature of the resulting suspension is then cooled from 40°C to 50°C.

The coagulated gelatinous particles are filtered, washed several times with acetone and then dried in a conditioned drier to give the gelatin-coated granules.

WHAT WE CLAIM IS:—

1. A process for the preparation of a medicinal agent for the treatment of hypercholesterolemia from the oil of marine animals characterised in that the oil of marine animals is added with an antioxidant and then purified by electrolytically reducing it in the presence of a neutral or alkaline solution of an electrolyte, and that the purified and stabilized oil is then used as the base material to prepare the medicinal agent in a conventional manner.

2. A process as claimed in Claim 1 in which the reduction is carried out in the presence of a powder of a metal selected from aluminium, magnesium and zinc.

3. A process as claimed in claim 1 or 2 in which the oil of marine animals is selected from the group consisting of sardine oil, herring oil, bonito oil, whale oil, mackerel oil, saury pike oil and oil of the internal organs of cuttlefish as well as residual oils which are obtained in the production of vitamin oils, such as liver oils of pollack, cod, shark, tunny and whale.

4. A process as claimed in claim 1 or 2 in which the antioxidant is selected from the group consisting of nordihydroguaiaretic acid, propyl gallate, butylated hydroxyanisole, butylated hydroxytoluene, and α-tocopherol.

5. A process as claimed in claim 1 or 2 in which the electrolyte is an aqueous solution of an inorganic base, for example sodium hydroxide, or of a salt of an organic acid for example sodium acetate, or of sodium chloride or sodium sulfate.

6. A process as claimed in claim 1 or 2 in which the electrolytic reduction of the marine animal oil is carried out in an electrolytic cell in which a diaphragm is provided between the anode chamber and cathode chamber.

7. A process as claimed in claim 1 or 2 in which the medicinal agent is a form of a capsule, an oily preparation or an emulsion.

8. A medicinal agent when prepared by the process as claimed in claim 1 or 2.

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